

Amendments to the Specification

The additions have been indicated by underlining.

Please replace the paragraph on page 3 lines 4-23 with the following amended paragraph:

[0009] Suitable examples for proteasome inhibitors thus in particular are threonine protease inhibitors, serine protease inhibitors and/or cysteine protease inhibitors, especially a peptide aldehyde, a peptide boronate, a peptide vinyl sulfone, a peptide epoxyketone, a lactacystine, a peptide alpha keto-aldehyde, an alpha-ketoamide, an indanone peptide, a polyalkylene aldehyde and/or a polyphenol, in particular a catechin-3-gallate, which can e.g. be extracted from green tea. Especially suitable as proteasome inhibitor are Z-Leu-Leu-Leu-al (MG132), Z-Ile-Glu(OtBu)-Ala-Leu-al (PSI) (SEQ ID No. 1), CEP1612, pyrazylcarbonyl-Phe-Leu-boronate (PS-341), dansyl-Phe-Leu-boronate (DFLB), morpholino-naphthylalanine-Leu-boronate (MG273), NIP-Leu₃-vinylsulfone (NLVS), Tyr-Leu₃-VS (SEQ ID No. 2), NIP-Leu-Leu-Asn-VS, Ada-Tyr-Ahx₃-Leu₃-VS (SEQ ID No. 3), Ada-Lys(Bio)-Ahx₃-Leu₃-VS (SEQ ID No. 4), Ac(Me)-Ile-Ile-Thr-Leu-EX (epoxomicin) (SEQ ID No. 5), dihydroeponemycin, lactacystine, clasto-lactacystine-beta-lactone (omuralide), PS-519, Ac-Leu-Leu-Nle-al (ALLN), 3,4-dichloroisocoumarine (DCI), 4-(2-aminoethyl)-benzenesulfonyl fluoride (Pefablock SC), TMC-95A, gliotoxin, (-)-epigallocatechin-3-gallate (EGCG), ritonavir, lovastatin, aclacinomicin A (Aclarubicin) and/or cyclosporin, which are all described more closely in Kisselev A. F. & Goldberg A. L. (2001, supra) and in FIG. 3, and wherein Z is a benzyloxycarbonyl group, al is an aldehyde group, VS is a vinyl sulfone group, NIP is a 3-nitro-4-hydroxy-5-iodophenylacetate group, and Bio is a biotin group. Particularly preferred of all these classes of compounds are threonine protease inhibitors and, from this, especially the compounds MG132, MG262, lactacystine and/or epoxomicine, above all MG132 and/or MG262.

Please replace the paragraph on page 4, line 25 with the following amended paragraph:

[0016] **FIG. 1C** shows the quantitative evaluation by means of computer-aided image analysis. The result here is a cardiac fibrosis being reduced by approximately 40% under MG132-treatment. Mean value \pm S.E.M. (standard error of mean=standard deviation/root of n), n=7-10, *:p<0,05 0.05.

Please replace the paragraph on page 4, line 30 with the following amended paragraph:

[0017] **FIG. 1D** shows the end-diastolic pressures (LVEDP) in the left ventricle of the rats with a remarkably reduced pressure level in the MG132-treated animals. Mean value \pm S.E.M., n=6-10, *:p<0,05 0.05, **:p<0,01 0.01.

Please replace the paragraph on page 5, line 1 with the following amended paragraph:

[0018] **FIG. 1E** shows the maximal pressure increase rate (dp/dtmax), which is significantly higher in the MG132-treated animals. Mean value \pm S.E.M., n=6-10, *:p<0,05 0.05, **:p<0,01 0.01.

Please replace the paragraph on page 5, line 4 with the following amended paragraph:

[0019] **FIG. 1F** shows the maximal pressure drop rate (dp/dtmin), which is increased by a factor of 2 under treatment with MG132. Mean value \pm S.E.M., n=6-10, *:p<0,05 0.05, **:p<0,01 0.01.

Please replace the paragraph on page 5, line 10 with the following amended paragraph:

[0021] **FIG. 2A** shows the dose-dependent inhibition of proliferation by MG132. The cells were stimulated with ~~0,1~~ 0.1 μ M and 1 μ M MG132 or ~~0,1~~ 0.1% DMSO and counted daily. Mean value \pm S.E.M., n=3.

Please replace the paragraph on page 5, line 19 with the following amended paragraph:

[0023] **FIGS. 3A and B** show the chemical structure of different proteasome inhibitors.

[Figure 3A - PSI: SEQ ID NO: 1; epoxomicin: SEQ ID NO: 5].

Please replace the paragraph on page 6, line 18 with the following amended paragraph:

[0030] MG132, ALLM and MG262 were purchased at Calbiochem.RTM. (San Diego, Calif., USA) and provided as 10 mM DMSO stock solutions. Cardiac fibroblasts (5×10^4 cells per ml) were inoculated in 24-well-plates. Adherent cells were either stimulated with MG132 (~~0,1~~ 0.1 and 1 μ M) or DMSO (~~0,1~~ 0.1%) in medium with 10% serum and further cultivated for 7 days. The medium was exchanged every second day. The proliferation was determined by counting the living cells in triplicate in daily intervals by means of the trypanblue exclusion test. This method allows for the distinction between living and dead cells: the dye trypanblue, which is added to the cells, can only enter into cells having a defective cell membrane, which are consequently stained blue. Living cells with an intact cell membrane are not stained.

Please replace the paragraph on page 7, line 21 with the following amended paragraph:

[0036] Male SH-rats of the same age were obtained from the Jackson Laboratory, Maine, USA. The animals were maintained according to the international guidelines for the keeping and use of laboratory animals. 6-week-old rats were treated for 12 weeks with MG132 (1 mg per kg body weight), ~~0,4~~ 0.1% DMSO as solvent control or with physiological saline (n=10 per group, daily intraperitoneal injection). The animals were fed with a high salt diet (drinking water with 2% sodium chloride). The blood pressure was measured weekly by means of the standard tail-cuff method. The blood values and the standard laboratory markers of the renal and hepatic functions were determined 6 weeks after the treatment and at the end of the study by means of standard methods. At the end of the study, the animals were anaesthetised by means of an intraperitoneal injection of a 20% urethane solution (~~0,9~~ 0.9 g per kg body weight) and the left-ventricular pressure parameters were determined as being described by Saragoca, M. et al. (1981) Hypertension, 3, 380-385. The organs were withdrawn, weighed and either shock-frozen in liquid nitrogen or embedded in paraffin for the histological analysis. Since no difference was observed between the DMSO-treated and the salt-treated SH-rats in respect to the above mentioned parameters, the DMSO-treated animals served as a control for the MG132-treatment.

Please replace the paragraph on page 8, line 4 with the following amended paragraph:

[0037] The data was determined in the form of mean values \pm S.E.M., if not otherwise indicated. The significance of the differences in the left-ventricular pressure parameters and in the quantification of the heart fibrosis was determined according to Student's T-test. For the cell proliferation test, the significance was determined by comparing the regression coefficients of MG132 in relation to the control group. An error probability of ~~p<0,05~~ 0.05 was considered as being significant. The software SPSS 9.0 was used for all statistical calculations.

Please replace Table 1 on page 8 with the following amended table:

	Control	1 mg/kg MG132
Systolic BD (mm Hg)	496,75 \pm 9,1 <u>196.75 \pm 9.1</u>	491,43 \pm 11,2 <u>191.43 \pm 11.2</u>
Body Weight (g)	286 \pm 36,6 <u>36.6</u>	280 \pm 15,3 <u>15.3</u>
Heart Weight (g)	1,28 \pm 0,07 <u>1.28 \pm 0.07</u>	1,18 \pm 0,07 <u>1.18\pm0.07</u>
HG/KG (mg/g)	4,64 \pm 0,61 <u>4.64\pm 0.61</u>	4,1 \pm 0,34 <u>4.1 \pm 0.34</u>

Please replace the paragraph bridging pages 8 and 9, with the following amended paragraph:

[0042] The prevention of cardiac fibrosis in MG132-treated SH-rats correlated well with the normal left-ventricular function, whereas the controls showed indications of a beginning left-ventricular dysfunction as shown in the FIGS. 1D-1F: The filling pressures

(LVEDP) were significantly lower (FIG. 1D: 15 ± 2 versus 5 ± 3 mm Hg, $p=0.017$ 0.017), the maximal pressure increase rate dp/dt_{max} (FIG. 1E: 8010 ± 538 versus 3375 ± 662 mm Hg, $p=0.003$ 0.003) and the maximal pressure drop rate dp/dt_{min} (FIG. 1F: -5046 ± 726 versus -2290 ± 422 mm Hg/s, $p=0.015$ 0.015)--which are parameters of cardiac inotropy and cardiac lusitropy--were more than twice higher in the MG132-treated SH-rats in comparison to the control SH-rats.

Please replace the paragraph on page 9, line 8 with the following amended paragraph:

[0043] In order to elucidate possible mechanisms, by which the proteasome inhibition may contribute in vivo to the reduced cardiac fibrosis, primary cardiac fibroblasts of the rat were treated with proteasome inhibitors. As illustrated in FIG. 2A, the treatment of cardiac fibroblasts with ~~0.1~~ 0.1 and 1 μ M MG132 induced a dose-dependent inhibition of proliferation. Moreover, the RNA-expression of collagen $I\alpha 2$ and $III\alpha 1$ was inhibited by MG132 in a dose-dependent manner by up to 73% and up to 91% (FIG. 2B). In contrast to this, the expression of collagen $I\alpha 1$ was unaffected. A second specific proteasome inhibitor, MG262, which is a boronate derivative of MG132, inhibited the collagen expression in a comparably effective manner (FIG. 2B) and thus proves the specificity of the proteasomal inhibition. The cathepsin inhibitor ALLM, which as a peptide aldehyde (ALLM-al) is structurally related to MG132, in contrast showed no effects on collagen expression (FIG. 2B).

Please replace the paragraph on page 12, line 31 with the following amended paragraph:

[0063] As shown in FIG. 6, cardiac fibroblasts have no active NF κ B complex under non-stimulated, basal conditions (see control bands). Under IL-1 β stimulation, an activation of NF κ B takes place, represented as band shift. The simultaneous treatment with 0,5 0.5 μ M MG132 took not less than 6 hours to lead to a small inhibitory effect on NF κ B, which however is in no way sufficient to explain the surprisingly strong reduction of the expression of MMP2 and MMP9 in case of the simultaneous application of MG132 and IL-1 β (see above, FIG. 4A). After 24 h, one observed a decrease of the IL-1 β -induced activation of NF κ B with and without MG132. The in vitro experiments thus show that the inventive inhibitory effects of proteasome inhibitors on the expression of collagens and MMPs are mediated independently from the inflammatory transcription factor NF κ B.

Please replace the paragraph on page 14, line 4 with the following amended paragraph:

[0068] In the prevention and therapy of fibroses according to the invention, a patient is given at least one proteasome inhibitor, preferably in a dose of approximately 0,5 0.5 μ g/kg body weight to approximately 0,5 0.5 mg/kg body weight, preferably in a dose of approximately 1 μ g/kg body weight to approximately 0,4 0.1 mg/kg body weight, preferably in a dose of approximately 0,04 0.01 mg/kg body weight to approximately 0,4 0.1 mg/kg body weight. These doses refer to all of the proteasome inhibitors mentioned in this specification, especially to the threonine protease inhibitors, in particular to MG132 and MG262. Preferably, at least one of the proteasome inhibitors mentioned at the beginning of this specification is administered, preferably MG132. For this aim, one preferably uses advantageous pharmaceutical formulations, which are familiar to the

expert, preferably solid and liquid medicinal preparations, preferably solutions for infusion, which preferably contain at least one advantageous pharmaceutical additive, which is known to the expert and which contributes to the shelf life and/or the reduction of side effects of the medical composition.